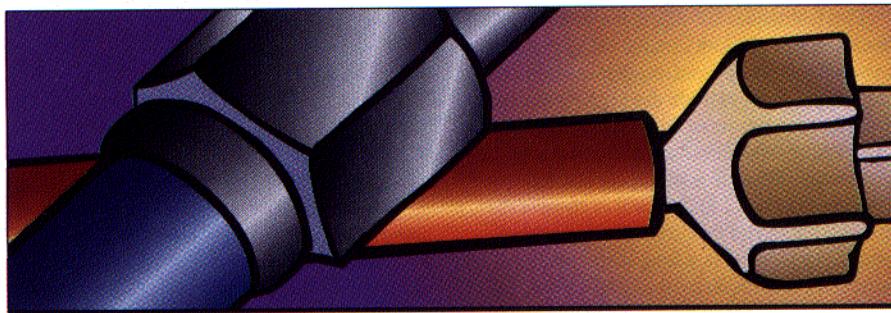


LC Troubleshooting



Useful or Useless? — Part II

John W. Dolan

Fact or fantasy? Do your liquid chromatography practices make good sense?

This "LC Troubleshooting" column is the second of a two-part series that examines practices in liquid chromatography (LC) methods that may or may not make good chromatographic sense. Last month I examined saturator columns, in-line filters, guard columns, and column voids (1). This month I will discuss a few more practices.

CLEANING FRITS

Q: What is the best way to clean a frit? When I try to clean the solvent inlet frit or a column frit by sonication, it isn't successful every time. Is there a special technique that I should use?

A: Cleaning a frit typically is an unwise use of your time, if you figure that the true cost of a chemist is in the range of \$200,000/year — approximately \$100/h — for many companies. Is it worth spending 15–20 min trying to extend the life of a \$10 inlet-line frit by cleaning it? Sometimes you might need to clean a frit, such as when you don't have a spare. However, proper planning should prevent this problem. The frit blockage may or may not be reversible. If frit cleaning is necessary, try sonication in dilute nitric acid (a 1:10 dilution with water) for a few minutes followed by sonication in a couple changes of high performance liquid chromatography (HPLC)-grade water. I don't recommend cleaning column frits by this technique, because it is difficult to determine which side should be placed next to the column packing. If the frit is reversed,

insoluble debris could wash onto the column and cause further problems.

STEPPING FLOW

Q: My boss insists that all changes in the operation of my LC system be made in a stepwise manner. For example, the flow rate must be stepped gradually from 0 to 2 mL/min when the system is started, and gradient methods must use a reverse gradient to return the column to the starting conditions. Do these practices really make the columns last longer?

A: In the early days of modern LC, columns were not packed as well as they are today, and column collapse was a common problem. Sudden changes in flow rate or pressure pulses from rotating an injection valve too slowly could cause the column packing to settle and thus form voids. Sometimes analysts observed the same problem when reequilibrating a column after a gradient run. With today's columns these problems rarely occur. Improvements in column preparation have resulted in columns that are much more stable than they were 20 years ago. So as a general rule, if the column is silica-based, it should be immune to sudden pressure changes of a reasonable magnitude such as that caused by turning on the pump at 2 mL/min. Nonsilica columns may be more susceptible to pressure shock, so when using a column such as a soft-gel size-exclusion column, analysts should check the column insert for any special handling recommendations. For the reequilibration of a column in a gradient method, the

important factor is the volume of solvent that passes through the column at the start of the method, not the manner in which the transition is made from the final to initial conditions.

PUMPING A COLUMN DRY

Q: Now and then I don't leave sufficient mobile phase in the reservoir for an overnight run, and when I come in the next morning, the pump has shut down and is displaying a low-pressure error message. Does this situation damage the column? If I have pumped out all the mobile phase from the column, can the column be used again?

A: I think all chromatographers encounter this problem now and then, whether or not they want to admit it. If you have pumped the solvent reservoir dry, it is unlikely that you caused any column damage. Remember that after the pump is full of air, it isn't going to pump air into the column. LC pumps are great for pumping liquids, but nothing happens if you try to pump air. So if you pump the reservoir dry, just reprime the pump and allow it to pump to waste for a few minutes to ensure that all the air is out of the system. Then you should be able to resume operation.

A more likely result is that the column will dry out if you forget to put the end plugs in the column when you store it or if the plugs

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are loose. Again, it is unlikely that the whole column will dry out. Perhaps a few millimeters at the end of the column will dry out, but it would take a long time for all the solvent to evaporate. Even if the column dries out, it is not necessarily ruined. Try pumping a thoroughly degassed, low-surface-tension solvent through the column to remove the air. For example, helium-sparged methanol would be a good choice. The low surface tension should aid in wetting the packing surface, and the degassed solvent should dissolve any trapped air as the solvent passes through the column. After the column is thoroughly wetted — for example, after 1 h of pumping at 1 mL/min — test the column to be sure it passes the column

manufacturer's specifications. If the column fails, it probably isn't worth spending any more time trying to revive it.

FILLING VOIDS

Q: What's the best way to fill a column void?

A: I think the best way to fill a void is to replace the column. Consider a few of the factors in this decision. A void forms at the top of the column for one of two reasons. If the column packing is crushed from excessive pressure shock, it will settle and form a void. Alternatively, the column packing can dissolve and create a void. Both of these causes result in changes in the surface chemistry of the column, so even if you could fill the void successfully, it is unlikely that the column chemistry would be the same after the repair. You could argue that a small void certainly wouldn't result in a major chemistry change; this argument may be true for some — but certainly not all — applications.

Another factor to consider is the cost of the column relative to the overall analysis cost. A figure of \$50/sample commonly is used to estimate the cost of analysis for pharmaceutical samples, and this amount corresponds roughly to the cost of having work performed by a contract laboratory. What makes up these costs? If the column costs \$400 and lasts for 800 samples, it contributes just \$0.50/sample, or 1% of the overall cost. Sample preparation can cost several times this amount — solid-phase extraction cartridges can cost \$2 each, plus labor is involved in sample preparation. A \$30,000 LC system analyzing 400 samples per week amortized over 3 years would contribute another \$0.50-\$1 per sample. Most of the \$50/sample cost is for labor — sample tracking, data analysis, and report writing. By

backup column is available. In these cases, filling the void may be worthwhile. One successful technique involves filling the void and then reversing the column (2) — this procedure seems to prevent the immediate recollapse of the bed that sometimes happens if the column is used in its original flow direction.

Two techniques for filling the void have been successful. Both involve using packing material recovered from another column of the same type. First, extrude the packing material by removing the outlet endfitting and pumping the packing out. Throw away the first and last centimeter or two to avoid using contaminated packing material. I find it easy to remove the column outlet and then extrude the packing onto a paper towel in a long strip, much like squeezing a toothpaste tube. One void-filling technique calls for mixing the packing with methanol to a paste-like consistency and then using a spatula to spread this mixture on the top of the voided column until it is even with the end of the tubing. Other users add more methanol to make a dilute slurry that can be dripped into the end of the column with a Pasteur pipette to fill the void. After the void is filled, rinse the endfitting and ferrule, put a new frit on the column and reassemble it. If you follow the directions of reference 2, reverse the column before reinstalling it.

PEEK TUBING AND FITTINGS

Q: Under what conditions can I use polyetheretherketone (PEEK) tubing and fittings?

A: PEEK can be used for most LC applications as a direct substitute for stainless steel for high-pressure connecting tubing and fittings. Analysts should consider two cautions. First, PEEK is incompatible with methylene chloride and tetrahydrofuran. Other LC solvents are OK, but the tubing becomes brittle after exposure to these two solvents. The solvents don't seem to dissolve the tubing or leach compounds out of it, but the brittleness does occur. I have seen cases in which tetrahydrofuran was used inadvertently with PEEK, and everything was OK until an analyst accidentally bumped the tubing and it snapped. The second consideration is that PEEK tubing has lower pressure limits than stainless steel. Stainless steel can be used at pressures as high as the pressure limits of the LC system (typically 6000 psi), whereas PEEK has limits closer to 4000 psi (consult the manufacturer's literature for exact specifications). However, most methods rarely exceed 3000 psi, so this pressure limitation impacts only a few types of analyses.

I like to use PEEK tubing whenever I'm frequently making and breaking connections. For example, between the injector and the column and between the column and detector. The added flexibility makes PEEK especially convenient for such applications. For connections that I want to make once and forget, I prefer stainless steel because it doesn't have the pressure or solvent limits of PEEK. For

example, the connections between the pump and autosampler are stainless steel.

Similar rules apply for PEEK fittings. For the most part, PEEK fittings are finger-tightened, so they are more convenient to use. And because little or no solvent contacts the fittings, you don't need to worry about solvent

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compatibility with PEEK nuts and ferrules. As with the tubing, however, the pressure limits of the finger-tightened fittings are a bit lower than stainless steel. If the pressure is too high, the tubing could slip in the fitting and cause a leak or unintended extracolumn dead volume. So I use PEEK fittings where connections are made and broken regularly such as at the column ends. PEEK fittings have the added advantage of easy adjustment for proper connection with different column brands. If stainless steel fittings are used for column connections, it is possible to make up the fitting in one column brand and have a small cavity in the fitting assembly when another column is used. Analysts avoid this problem with finger-tightened PEEK fittings because they are easy to adjust for each new application. As with the stainless steel tubing, however, I prefer stainless steel nuts and ferrules for parts of the system I want to connect once and then ignore.

CONCLUSIONS

This month I have described LC techniques such as stepping flow rates and filling column voids, practices that were justified when columns were not as well made as they are today. Some practices, especially those intended to extend column life, should be considered from an economic standpoint. It makes little sense to spend more trying to fix a defective part than it would to replace the part with a new one. Similarly, spending your valuable time for a marginal economic return doesn't make much sense.

REFERENCES

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how much does the repair of the column extend the expected column life? Even if you could double the lifetime of the column by filling a void, it would have little impact on the overall cost of analysis. It is better to put your efforts into reducing the more costly portions of the process.

With all that said, sometimes filling a column void makes sense, such as when you are using a chiral column or other specialty column that might cost 4-5 times as much as a standard analytical column. Another case is when you have to analyze a sample set and no